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Molasses injection as a MEOR strategy:

Enrichment incubations of brine/oil from North Sea Oil Field

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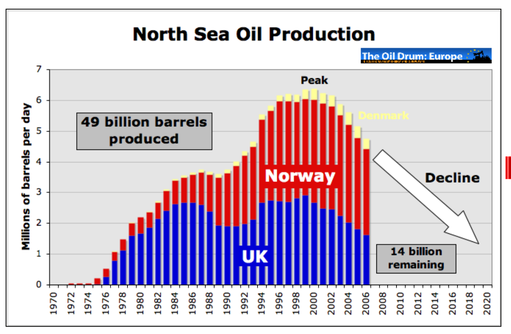
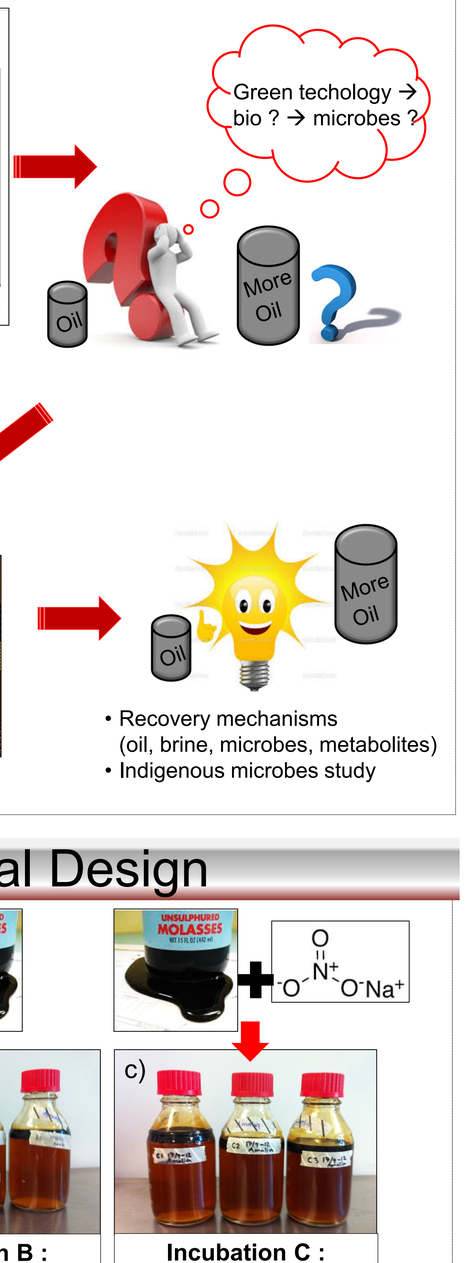
Background and Objectives





- Recovery mechanisms (oil, brine, microbes, metabolites)
- Indigenous microbes study

Experimental Design

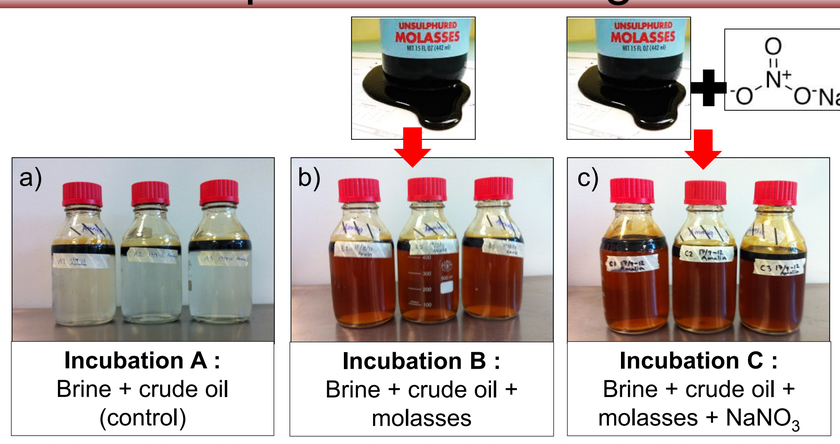
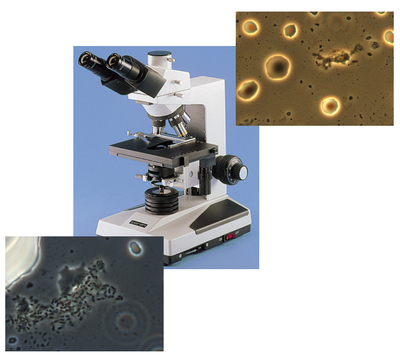
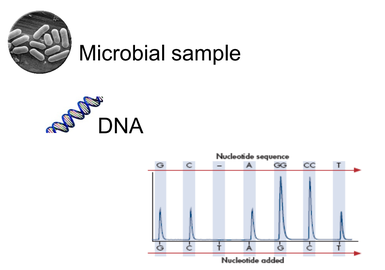


Figure 1. Anaerobic enrichment tests by using brine and crude oil from the North Sea oil field for 28 days at 55°C. Three different conditions on the enrichment test: (a) incubation A (control) – no nutrient addition, (b) incubation B with addition of 2% molasses and (c) incubation C with addition of 2% molasses and 2.5 mM nitrate.

- Cell hydrophobicity



- How many microbes ? qPCR
- Which microbes? pyrosequencing



- Sugar hydrolysis and metabolite production
- Oil characteristics (viscosity, density, and total weight fraction)
- Interfacial tension (oil vs. brine)
- Emulsion

Acknowledgement

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- Nordic Sugar is acknowledged for providing the molasses for the experiment.

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Results

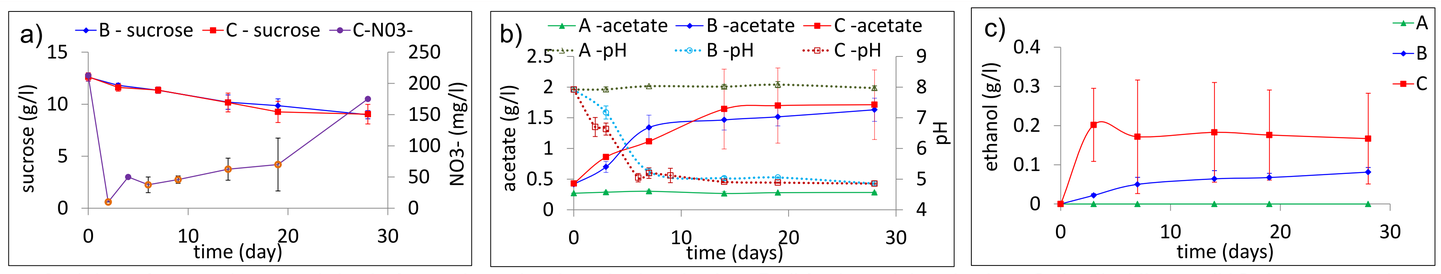


Figure 2. (a) molasses (sucrose) hydrolyses by microbes in incubation B (blue) and incubation C (red). Nitrate (NO_3^-) was monitored in incubation C, NO_3^- was added at day 2, 6, 9, 14, 19 (orange circle) to keep NO_3^- concentration at 212.5 mg/l (2.5mM), (b) acetate production and brine pH during incubation, (c) ethanol production during incubation.

- Sucrose hydrolyses (incubations B and C) – presence of fermentative microbes. Nitrate was consumed continuously in incubation C (Fig. 2a).
- Acetate production – pH decrease (incubations B and C, Fig. 2b) and ethanol production (Fig. 2c).
- No production of succinate, lactate and glycerol on all incubations.

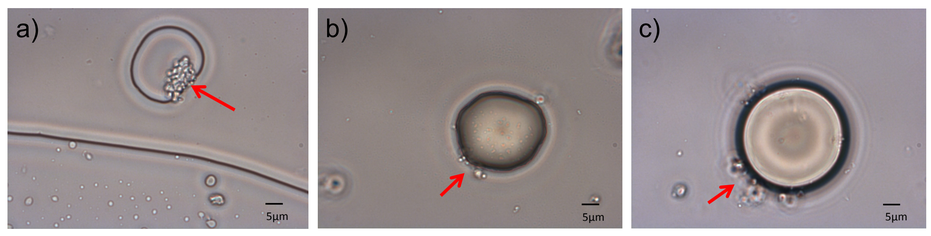


Figure 3. Microbes were present in the oil-brine interphase in all incubations (red arrow). (a) In incubation A, without molasses, microbes had a tendency to form aggregates. (b, c) In incubation B and C, with addition of molasses, microbes are present as single cell (loose particle).

Table 3. Interfacial tension (IFT) between the brine and the crude oil in incubation A, B and C

Sample	T0	T28 - with biomass	T 28 w/o biomass
A	19.29 ± 0.12	17.70 ± 1.48	15.20 ± 0.32
B	18.79 ± 0.18	2.34 ± 0.15	21.65 ± 0.15
C	18.60 ± 0.09	2.96 ± 0.43	22.60 ± 0.88

- In chalk it is important that microbes are present as single cells (hydrophilic) to prevent blocking of pore throat.
- No significant change in oil characteristics: viscosity, density and composition (total weight fraction) in all incubations.
- Formation of gasses, emulsions and smaller oil droplets in incubations B and C.
- IFT decreases when microbes are present in oil-brine interphase (Table 3).

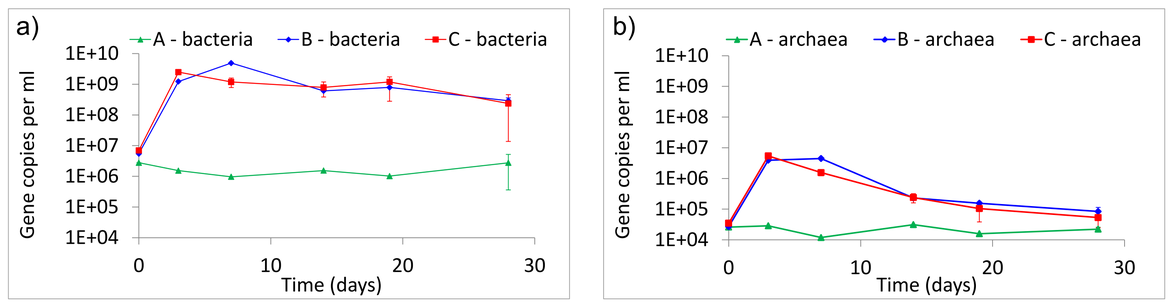


Figure 4. Abundance of 16S rRNA bacteria and 16S rRNA archaea gene copies in incubations A, B and C.

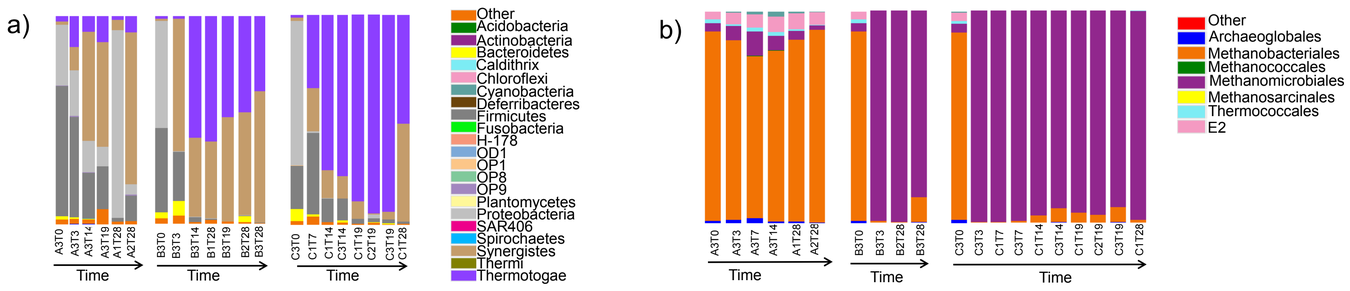
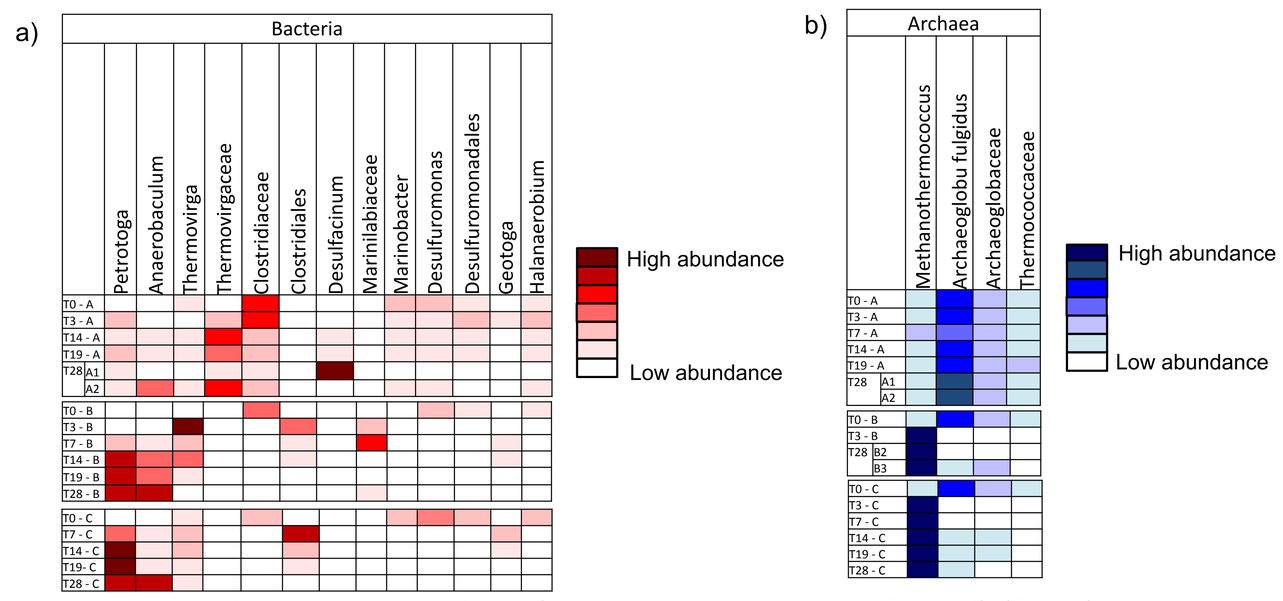


Figure 5. Development in (a) bacterial community at phylum level and (b) archaeal community at organism level in incubation A, B and C.

Table 2. Summary of heat map of the most abundant microorganisms based on sequencing of the16S rRNA gene of (a) bacteria and (b) archaea



- Microbial growth was stimulated by addition of molasses in incubations B and C (Fig. 4).
- Sequencing revealed the population dynamics of the microbial community (Fig. 5 and Table 2).

Conclusion

- Production of fermentation products : acetate, ethanol and gas from sugar hydrolyses.
- No significant change in oil characteristics; but formation of emulsion, smaller oil droplets and reduction of IFT were observed due to microbial biomass when molasses was added (incubation B and C).
- Addition of molasses made microbe cells hydrophilic - prevent microbes to form aggregates.
- Molasses stimulated microbial growth in incubations B and C, as shown by increase in cell number of both bacteria and archaea (qPCR results).
- Pyrosequencing revealed that molasses stimulated the growth of *Petrotoga* and *Anaerobaculum*.
- Nitrate was consumed continuously and *Petrotoga* was favoured over *Anaerobaculum* in the presence of nitrate (incubation C).
- The growth of methanogenic archaea was potentially fueled by products from fermenting bacteria.